Complementarity among four highly productive grassland species depends on resource availability

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Abstract: Positive species richness-productivity relationships are common in biodiversity experiments, but how resource availability modifies biodiversity effects in grass-legume mixtures composed of highly productive species is yet to be explicitly tested. We addressed this question by choosing two grasses (Arrhenatherum elatius and Dactylis glomerata) and two legumes (Medicago × varia and Onobrychis viciifolia) which are highly productive in monocultures and dominant in mixtures (the Jena Experiment). We established monocultures, all possible two- and three-species mixtures, and the four-species mixture under three different resource supply conditions (control, fertilization, and shading). Compared to the control, community biomass production decreased under shading (-56 %) and increased under fertilization (+12 %). Net diversity effects (i.e., mixture minus mean monoculture biomass) were positive in the control and under shading (on average +15 and +72 %, respectively) and negative under fertilization (-10 %). Positive complementarity effects in the control suggested resource partitioning and facilitation of growth through symbiotic N2 fixation by legumes. Positive complementarity effects under shading indicated that resource partitioning is also possible when growth is carbon-limited. Negative complementarity effects under fertilization suggested that external nutrient supply depressed facilitative grass-legume interactions due to increased competition for light. Selection effects, which quantify the dominance of species with particularly high monoculture biomasses in the mixture, were generally small compared to complementarity effects, and indicated that these species had comparable competitive strengths in the mixture. Our study shows that resource availability has a strong impact on the occurrence of positive diversity effects among tall and highly productive grass and legume species.

DOI: https://doi.org/10.1007/s00442-016-3587-4

Complementarity among four highly productive grassland species depends on resource availability

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MS details: 245 words in abstract, 6593 words in full text, 5 figures, 1 table, Supplementary Material[1]

[1] Author Contributions: CR, EDS, BS and OK conceived and designed the experiment. CR and OK performed the experiment. CR analyzed the data and wrote the manuscript; EDS and BS discussed the results and contributed to writing the manuscript, OK provided editorial advice.

Abstract

Positive species richness–productivity relationships are common in biodiversity experiments, but so far it has not been explicitly tested how resource availability modifies biodiversity effects in grass–legume mixtures composed of highly productive species. We addressed this question choosing two grasses (*Arrhenatherum elatius, Dactylis glomerata*) and two legumes (*Medicago x varia, Onobrychis viciifolia*), which are highly productive in monocultures and dominant in mixtures (Jena Experiment). We established monocultures, all possible two- and three-species mixtures and the four-species mixture at different resource supply (control, fertilization, shading). Compared to the control, community biomass production decreased under shading (–56%) and increased under fertilization (+12%). Net diversity effects (i.e. mixture–mean monoculture biomass) were positive in the control and under shading (+15% and +72% respectively) and negative under fertilization (–10%). Positive complementarity effects in the control suggested resource partitioning and facilitation of growth through symbiotic N_2 fixation of legumes. Positive complementarity effects under shading indicated that resource partitioning is also possible when growth is carbon limited. Negative complementarity effects under fertilization suggested that external nutrient supply depressed facilitative grass–legume interactions due to increased light competition. Selection effects, which quantify the dominance of species with particularly high monoculture biomass in the mixture, were generally small compared to complementarity effects and indicated that the species had comparable competitive strength in mixture. Our study shows that resource availability has strong impacts on the occurrence of positive diversity effects among tall and highly productive grass and legumes species.

Keywords: biodiversity effects, fertilization, grass, legume, shading

Introduction

Experimental studies have demonstrated that biodiversity and ecosystem functions, such as aboveground primary productivity, are predominantly positively related (Hooper et al. 2005; Cardinale et al. 2012). Explanations for increased biomass production in mixtures have been controversially discussed and centre around two hypotheses, which are not mutually exclusive. The complementarity effect hypothesis is based on the assumption that more diverse communities use available resources more completely due to greater complementarity in the acquisition and use of resources or more opportunities for facilitative interactions (Tilman et al. 1997). The so-called sampling (or selection) effect hypothesis states that in randomly constructed communities, species-rich communities have a higher probability of containing a species with particularly large individual effects on biomass production (Huston 1997; Tilman et al. 1997). However, the physiological species-specific mechanisms underlying both hypotheses are poorly understood.

Natural grassland communities are usually a mixture of few tall-growing species, which make up the largest fraction of community biomass, and a larger number of small species contributing a minor proportion to community biomass (Grime 1998). Light and nutrient availability are the most limiting resources for plant growth in temperate grasslands in humid regions. Competition for light is asymmetric due to the unidirectional supply of incoming radiation. Taller growth warrants a disproportionate advantage in stands of vegetation by simultaneously increasing light capture and shading smaller competitors (Schwinning and Weiner 1998). Although competition for soil resources is generally thought to be size-symmetric, it also has been demonstrated that belowground competition may be size-asymmetric if soil resources are heterogeneously distributed (Rajaniemi 2003).

Most biodiversity experiments have focused on biodiversity–ecosystem functioning relationships under particular environmental conditions and effects of varying environmental conditions have been addressed in only some experimental studies showing that varying

levels of nutrient availability (e.g. Reich et al. 2001; Fridley 2002; He et al. 2002; Lanta and Lepš 2007; Nyfeler et al. 2009; Wacker et al. 2009) may impact diversity–productivity relationships. For example, Nyfeler et al. (2009) have shown that in fertilized agricultural grasslands composed of legumes and grasses, diversity–productivity effects were reduced and rapidly disappeared at high levels of N fertilization. Although it is common knowledge that plant productivity and plant–plant interactions are strongly controlled by resource availability (Grime 2001) and it has been widely acknowledged that the close interaction between carbon and nutrient metabolism requires a balance between nutrient and light supply for growth, we are aware of only a single biodiversity experiment manipulating soil resources by fertilizer addition and light availability by shading and this experiment used annual forb species (Fridley 2003). This study showed that overyielding, i.e. greater biomass in mixtures compared with monocultures, was highest under fertilization and full light.

Although it has been demonstrated that particular species or functional groups are important for the observed extent of complementarity and selection effects, to our knowledge biodiversity experiments never have been restricted to tall and dominant species. Here, we present results from an experiment specifically testing for diversity effects among four grassland species (two grasses, two legumes) with tall growth stature, high productivity in monoculture and the capability for fast establishment (Heiße et al. 2007), which have been identified as dominant species in the mixtures of a large biodiversity experiment (Jena Experiment) over several years (Roscher et al. 2005, 2011). However, these species do not occur in combination in the randomly assembled communities of the Jena Experiment (Roscher et al. 2004). We grew monocultures and all possible mixtures of these species with three replicates, which were assigned to different treatments after one year of growth to manipulate the availability of the most important resources limiting plant growth in temperate grasslands (control vs. fertilization vs. shading).

Fertilizer addition increases the availability of soil resources, but usually it also enhances competition for light due to increased plant biomass and canopy shading. Manipulation of light availability by shading is likely to decrease productivity due to light limitation, but it does not necessarily increase competition for light, when canopy shading declines with decreased plant biomass. In addition, lower productivity under low-light conditions may attenuate competition for soil resources due to lower demands for plant growth. Both manipulations, fertilization and shading, are likely to favor the growth of grasses over legumes due to the high energetic costs for N_2 fixation reducing legume performance in low-light circumstances (Vitousek and Howarth 1991). Beneficial effects of legumes on the community-level productivity of grass–legume mixtures are most likely under conditions where nutrients are the major growth-limiting resource and symbiotic N_2 fixation of legumes facilitates the growth of grasses in the mixtures. We measured aboveground biomass production as well as plant and soil variables related to nitrogen acquisition to test the following specific hypotheses:

(1) Selection effects among tall and dominant grassland species are small compared to complementarity effects because they have a similar competitive strength as mixture species.

(2) Complementarity effects are positive in the control without resource manipulation due to nutrient partitioning and facilitation of grasses through N_2 fixing legumes.

(3) Complementarity effects become negative under fertilization when external nutrient supply supress facilitative grass–legume interactions due to increased light competition

(4) Complementarity effects do not occur under shading because growth is generally limited by reduced light availability.

These hypotheses imply resource availability strongly impacts the possibility for positive diversity effects among tall and dominant grass and legume species.

Material and methods

123

124 **Experimental design**

125 The experiment was established at the field site of the Jena Experiment (Roscher et al. 2004)
126 located in the floodplain of the river Saale north of the city of Jena (Germany, 50°55`N,
127 11°35`E, 130 m a.s.l.). The area around Jena has a mean annual air temperatures of 9.3°C, and
128 annual precipitation is 587 mm (Kluge and Müller-Westermeier 2000). The soil is a loamy
129 Eutric Fluvisol. The experimental area was used as a fertilized agricultural field for about 40
130 years before sowing a grass mixture in spring 2002. After two years, in autumn 2004, the area
131 was ploughed to 30 cm depth and covered with a black perforated foil (Aquafol, Hermann
132 Meyer KG, Rellingen, Germany) until 2007. In spring 2008, the area was harrowed and raked
133 several times to remove emerging weed seedlings before sowing the experiment on plots of 2
134 × 2 m size on 05-May-2008.
135 We selected two perennial legume (*Medicago x varia* Martyn and *Onobrychis viciifolia*
136 Scop.) and two perennial grass species (*Arrhenatherum elatius* (L.) J. et C. Presl and *Dactylis*
137 *glomerata* L.) for our experiment. These experimental species, which were among the tallest
138 and most productive species in the Jena Experiment, were established as monocultures, all
139 possible 2- and 3-species-combinations and the full 4-species mixture. The experiment was
140 arranged in three blocks, each comprising a randomly positioned replicate of each
141 monoculture, 2- and 3-species mixture and two replicates of the 4-species mixture (= 16 plots
142 per block and 48 plots in total). Seeds were purchased from the same commercial supplier as
143 for the establishment of the Jena Experiment (Rieger-Hofmann GmbH, Blaufelden-
144 Raboldshausen, Germany). Plots were sown at total density of 1000 viable seeds per m^2 in a
145 substitutive design, in which constant total density was achieved by reducing sowing densities
146 of individual species according to the number of species in the mixture. Number of sown
147 seeds was adjusted for germination rates from laboratory tests. After mowing in September
148 2008, all plots were re-sown with a total density of 500 viable seeds per m^2 to mimic a diverse

natural age structure for all species. In the second year, blocks were allocated to experimental treatments manipulating (1) light availability through shading, (2) nutrient availability through fertilization, and (3) control. In the fertilizer treatment, fertilizer was applied as commercial NPK-pellets (slow release NPK fertilizer 150:65.4:124.5 kg ha^{-1} a^{-1}) divided into two equal portions in early spring (31-Mar-2009) and after the first mowing (21-Jun-2009). The amount of fertilizer is equivalent to usual fertilizer intensities in agriculturally managed semi-natural grasslands in Europe (Olff et al. 1990). In the shade treatment a reduction of photosynthetically active radiation was achieved by two layers of green shading cloth (polyethylene, aperture size 2 × 10 mm, Hermann Meyer KG, Rellingen, Germany) fixed on a roof (2.5 m height) and covered on all sides; this was installed in spring (14-Apr-2009). To assess the effects of shading on climatic conditions a weather station was established under the roof to measure the following variables: air temperature and relative humidity (KPK1/5-ME Humidity/Temperature Sensor, Mela Sensortechnik, Mohlsdorf-Teichwolframsdorf, Germany), photosynthetic active radiation (LI-90SA Quantum Sensor, LI-COR, Lincoln, Nebraska, USA) at 2 m height, precipitation (Precipitation Transmitter, Thies GmbH, Göttingen, Germany) and soil moisture at 8 cm, 16 cm, and 32 cm depth (ThetaProbe ML2X, Delta-T Devices Ltd., Cambridge, UK) and stored with a data logger (CR10X, Campbell Scientific, Logan, USA). The comparison with data from the central weather station of the Jena Experiment field site showed that shading reduced photosynthetically active radiation by 82%, precipitation by 40%, while soil moisture was increased on average by 20% in 8 and 16 cm depth and 40% in 32 cm depth. Air temperature and relative humidity did not deviate from ambient conditions (see Online Resource, Figs. S1 and S2).

Plots were regularly weeded to remove species not belonging to the sown species combinations. According to the typical management of extensively used hay meadows and the usual management of the Jena Experiment (Roscher et al. 2004), plots were mown in early June and September 2009 and mown plant material was removed.

175

176 **Data collection**

177 Aboveground plant biomass was harvested in one sample quadrat (50 × 50 cm size) in the
178 central area of each plot at estimated peak biomass before mowing on 03-Jun-2009 and 02-
179 Sep-2009. Plant material was clipped 3 cm above ground level and sorted to species,
180 separating unsown species and detached dead material. Samples were dried at 70°C (48h) and
181 weighed. Dry plant material per plot and species was shredded and a sub-sample was ground
182 with a ball mill to a fine powder. Approximately 20 mg were used for elemental C and N
183 analyses with an elemental analyzer (Vario EL Element Analyzer, Elementar, Hanau,
184 Germany).
185 During the spring harvest, material of fully developed leaves of all species was sampled in all
186 plots to determine N-isotope ratios. Isotope ratios were determined from approximately 3 mg
187 dried and finely milled leaf material with an isotope-ratio mass spectrometer (Delta C
188 prototype IRMS, Finnigan MAT, Bremen, Germany). The $\delta^{15}N$ values were calculated
189 relative to the international standard, the atmospheric nitrogen isotope ratio:

190 $$\delta^{15}N = (R_{sample}/R_{standard} - 1) * 1000 \quad \text{(eqn. 1)}$$

191 where R represents the molar ratio of $^{15}N/^{14}N$ in a sample. The $\delta^{15}N$ values of actively N_2
192 fixing legumes is close to the atmospheric $\delta^{15}N$ signal (~0 ‰) because the fractionation
193 of N isotopes in during symbiotic N_2 fixation is very small. Plants depending on soil
194 nitrogen show generally higher $\delta^{15}N$ values. Variation in the natural abundances of stable
195 ^{15}N isotopes in non-legume species may roughly indicate shifts in the uptake of different N
196 sources or the supply of depleted legume-derived N in communities with legumes (Högberg
197 1997).
198 Soil samples were taken at 21–Jun-2009 (after first mowing and before the second
199 fertilization) and 13-Oct-2009. Three soil cores of 1 cm diameter (0–15 cm depth) were
200 sampled in each plot and pooled. The fresh soil samples were sieved (2 mm mesh size) and

visible root parts were removed before extraction with 1M KCl (50 ml on 5 g soil material). The filtered extracts were frozen until analysis for nitrate and ammonium with a Continuous Flow Analyzer (SAN Plus, Skalar, Erkelenz, Germany). Inorganic nitrogen concentrations were expressed as µg per g dry soil after determining the water content of the soil samples gravimetrically (72 h, 105 °C) and averaged across sampling dates to compare between different resource manipulation treatments.

Calculations of overyielding

Relative Yield Total (RYT, de Wit and van den Bergh 1965) was calculated as a measure of overyielding in relative terms. The relative yield (RY_i) of a species i with respect to biomass is the quotient of a species` mixture biomass (O_i) and the monoculture biomass (M_i) of this species. The RYT of a mixture is the sum of the RYs of all component species. RYT > 1 indicates overyielding. The RYT is directly linked to "non-transgressive overyielding" quantified by the proportional index D_{mean}

$$D_{mean} = (O_T - \overline{M})/\overline{M} \quad \text{(eqn. 2)},$$

where O_T is the observed biomass of a given mixture, and $\overline{M}$ is the average monoculture biomass of all species in this mixture ($\overline{M} = M_i/N$; N = species richness); i.e. D_{mean} indicates whether a mixture outperforms the average of its component monocultures (D_{mean} = RYT-1; Loreau 1998). "Transgressive overyielding" ($D_{max} > 0$), indicating a higher biomass production of a mixture compared to the most productive monoculture of all species in this mixture (M_{max}), was calculated by replacing $\overline{M}$ by M_{max} in (eqn. 2) (Loreau 1998).

The ratio of observed over expected relative yields of individual species corrected for sowing proportions (i.e. multiplied with species richness N) were used to compare their performance and contribution to overyielding. A value of (N * RY_i) > 1 suggests that the species performs better in mixture than in monoculture, a value of N * RY_i = 1 indicates that a species has the

same performance in mixture and monoculture and (N * RY_i) < 1 indicates that the species performs worse in mixture than in monoculture.

To evaluate whether mixed communities depleted soil mineral nitrogen (ammonium and nitrate) more efficiently than monocultures, the proportional index D_{mean} (Loreau 1998) was modified by taking O_T as the observed soil mineral nitrogen in mixture, and $\overline{M}$ as the expected soil mineral nitrogen based on monocultures of the component species weighted by the biomass proportion of each species in the mixture. Values of $D_{mean} < 0$ indicate that mixtures reduce soil mineral nitrogen to lower levels than it would be expected from the component monocultures suggesting complementary resource use (Palmborg et al. 2005).

Furthermore, the additive partitioning method (Loreau and Hector 2001) was applied to assess diversity effects on biomass production. According to Loreau and Hector (2001), the net diversity effect (NE) on biomass of a given mixture is the difference between the observed total mixture biomass and the expected total mixture biomass based on the average of the monoculture biomass of the component species. The net diversity effect is the sum of two effects generated by species interactions in mixture, selection effects (SE) and complementarity effects (CE)

$$NE = SE + CE \qquad \text{(eqn. 3).}$$

Selection effects (SE) are quantified as the standard statistical covariance

$$SE = N \operatorname{cov}(\Delta RY, M) \qquad \text{(eqn. 4),}$$

where N is the number of species in mixture, M is a species` monoculture biomass, and ΔRY is the difference between the observed relative yield and the expected relative yield (i.e. its sown proportion $1/N$). Positive (negative) selection effects occur if species with higher-than-average (lower-than-average) monoculture biomass dominate mixtures. Complementarity effects (CE) measure to which degree all species similarly contribute to a higher (or lower) total mixture biomass than the average monoculture and is calculated as

$$CE = N\overline{\Delta RY}\,\overline{M} \qquad \text{(eqn. 5),}$$

where $\overline{\Delta RY}$ is the average ΔRY of all species in the mixture and $\overline{M}$ is the average monoculture biomass of all species in this mixture. Positive complementarity effects occur when increases in biomass of some species are not completely compensated by decreases in others and may indicate niche differentiation such as resource partitioning.

All calculations were done separately for each resource treatment.

Statistical analyses

Linear mixed-effects models using the *lmer* function in the library *lme4* (Bates et al. 2012) of the statistical software R (version 3.0.2, R Development Core Team, http://www.R-project.org) were used to analyse the influence of the experimental factors on the measured variables. Community-level variables were analysed starting from a constant null model with mixture identity as random effect. Then the fixed effects Resource (as a factor with three levels: control, fertilization, shading), sown species richness (SR; as a log-linear term) and the interaction between these terms (Resource × SR) were entered stepwise. In alternative models the presence-absence of each species (fitted after SR) and its interaction with Resource (fitted after Resource × SR) was included to test for species identity effects. In analyses of species-level variables mixture and plot were modelled as random effects, and species identity (Species ID) as well as the interaction Resource × Species ID were fitted as additional fixed effects. The maximum likelihood method (ML) and likelihood ratio tests (Chi^2) were applied to assess model improvement and the statistical significance ($\alpha \leq 0.05$) of the fixed effects. The *glht* function in the R library *multcomp* (Hothorn et al. 2009) was used to identify differences between the resource treatments and species (in analyses of species-level variables) with Tukey`s HSD test in models fitted with the restricted maximum likelihood method (REML). Separate analyses of variance (ANOVA) were conducted for each resource treatment for measures of overyielding and diversity to test grand means (i.e. overall means) against hypothetical values.

If necessary, data were log-transformed to meet the assumptions of linear mixed-effects models. Because the resource treatments had to be applied at block level, it is conceivable that resource treatment effects to some extent reflect block effects. This could not be avoided and is here mentioned as a caveat. Another shortcoming of our experimental design (and many other biodiversity experiments) is the lack of identical replicates of each species combination in the different resource treatments, which would allow to test for mixture identity effects and a more robust estimate of diversity effects.

Results

Community biomass production

Community biomass production varied with resource availability (Table 1; Fig. 1a). Averaged across species-richness levels, biomass production under fertilization was higher and biomass production under shading was lower than in the control (mean ± s.d.; control = 1195 ± 45 g_{dw} m^{-2}, fertilized = 1350 ± 48 g_{dw} m^{-2}, shaded = 503 ± 42 g_{dw} m^{-2}). On average, community biomass production increased with species richness, but species richness effects depended on resource availability (significant interaction Resource × SR; Table 1). Positive species-richness effects on community biomass production were larger under shading than in the control, while increasing species richness did not increase community biomass production under fertilization (Table S1). The presence/absence of *A. elatius*, *D. glomerata* or *M x varia* did not affect community-level biomass production, while the effects of *O. viciifolia* were dependent on resource availability (significant interaction Resource × Ov; Table 1). The presence of *O. viciifolia* had positive effects on community biomass production in the control and under fertilization, while having negative effects under shading (Table S1).

Non-transgressive and transgressive overyielding

Relative yield totals (RYTs) differed under varying resource availability (Table 1). The RYTs were smaller under fertilization and greater under shading than in the control (Fig. 1b). The overall mean of RYTs across all species-richness level were >1 (i.e. D_{mean} >0) in the control and under shading (mean ± s.d.; control = 1.186 ± 0.152, shaded = 1.608 ± 0.413) indicating non-transgressive overyielding. Under fertilization the overall mean of RYTs was <1 (0.877± 0.154). The RYTs did not change with increasing species richness. The presence of *D. glomerata* had negative effects RYTs (Table 1), which varied with resource availability. Under fertilization and shading the RYTs were smaller in mixtures with *D. glomerata* than in mixtures without this species, while this was not the case in the control (Table S1).

Transgressive overyielding (D_{max}) also varied with resource availability, but on average mixtures did not outperform the best monoculture in the control and under shading or even underyielded under fertilization (D_{max} <0; Table 1, Fig. 1c).

Net diversity, complementarity and selection effects

Resource availability also influenced net diversity effects (NE), complementarity effects (CE) and selection effects (SE) (Table 1). NE and CE for biomass production did not differ between the control and the shade treatment and were positive (Fig. 1d, e). Due to the lower productivity level under shading net diversity effects increased biomass in the mixtures by 72% under shading. The average increase in biomass amounted to 15% in the control. NE and CE were negative under fertilization, i.e. on average net diversity effects decreased biomass by 10%. SE for biomass production were small compared to CE (Fig. 1f); the SE were negative in the control (–4%), and positive under shading (+15%) and fertilization (+2%). Irrespective of the resource treatment, NE, CE and SE did not change with species richness. The presence/absence of *D. glomerata* fitted after species richness decreased NE and CE, while the presences/absences of other species had no additional effects on NE, CE or SE (Table 1). However, effects of *D. glomerata* varied with resource availability. The species had

strong negative effects on NE and CE under fertilization and mainly caused the negative NE and CE in this treatment (Table S1). The NE and CE were also smaller in mixtures with *D. glomerata* than without this species under shading, while *D. glomerata* tended to have positive effects on NE and CE in the control (Table S1). Dependent on resource availability, the presence-absence of *D. glomerata* also influenced SE. The SE effects tended to be more positive in mixtures with *D. glomerata* in the control and under fertilization, while SE were lower in mixtures with *D. glomerata* than without this species under shading (Table S1).

Species-level biomass production and relative yields

On average the grass species (*A. elatius, D. glomerata*) and the legume *M. x varia* produced more biomass than the legume *O. viciifolia* (Fig. 2a). Differences in biomass production among the grass species and the legume *M. x varia* depended on resource availability (Online Resource, Table S2). In the control, species biomass decreased in the order *A. elatius*, *M. x varia*, *D. glomerata*. In contrast, *D. glomerata* was more productive than *M. x varia* under fertilization (Fig. 2a). Under shading, biomass of *D. glomerata* and *A. elatius* did not differ and the grasses were more productive than the legume *M. x varia*.

Relative yields (RY) of individual species also varied with resource availability (Table S2; Fig. 2b). *Arrhenatherum elatius* had RY>1 in the control and under shading, but not under fertilization. *Dactylis glomerata* achieved RY>1 only under shading. The legume species did not achieve RY>1; and *O. viciifolia* even underyielded (RY<1) in the control and under fertilization. In general, increasing species richness did not affect species-level RY with the exception of positive species richness effects on RY of *A. elatius* in the control (analyses not shown).

Shoot nitrogen concentrations and leaf $\delta^{15}N$

Shoot nitrogen concentrations (N_{Shoot}) varied dependent on resource availability, while

increased species richness did not affect N_{Shoot} (Table S1). On average, N_{Shoot} was lowest in the control, slightly larger under fertilization and largest under shading (Fig. 3a). N_{Shoot} of the legume species were higher than in the grass species. N_{Shoot} were not different between the grass species and between the legume species except for lower N_{Shoot} of *M. x varia* than *O. viciifolia* under shading (Fig. 3a).

The $\delta^{15}N$ measured in leaf samples collected in spring before first mowing showed variation with resource availability. On average values under fertilization were reduced compared to the control and shading, but the effects of resource treatments depended on species identity (Table S2). Increased species richness did not alter leaf $\delta^{15}N$. Although leaf $\delta^{15}N$ of legumes were larger than zero in all samples, legume species (except for *M. x varia* under fertilization) had generally lower leaf $\delta^{15}N$ than the grass species suggesting that a portion of legume N was derived from symbiotic N_2 fixation (Fig. 3b). Additional analyses testing for differences in leaf $\delta^{15}N$ between grasses and legumes dependent on resource availability showed that differences between functional groups were largest in the control and smallest under fertilization (estimated coefficients from linear mixed-effects models: control = 3.62 ‰, fertilization = 1.02 ‰, shading = 2.07 ‰).

Soil mineral nitrogen concentrations

Soil mineral nitrogen concentrations (N_{min}) differed dependent on resource availability (Fig. 4a; Online Resource, Table S3). N_{min} had the lowest concentrations in the control, while N_{min} was largest under shading. Effects of increased species richness on N_{min} were not significant. The presence of *D. glomerata* led to a decrease in N_{min}, while the presence of *O. viciifolia* caused an increase. Observed over expected mineral soil nitrogen concentrations (D_{mean}) also varied with resource availability. The overall means across all species-richness levels did not differ between the control and the fertilized treatment, and both had values significantly lower than zero (Fig. 4b). Values observed under shading did not differ from expected values.

382

383 ## Discussion

384 Biodiversity experiments have been repeatedly criticized for their random scenarios of species
385 loss ignoring the impact of environmental and biotic filters which constrain species diversity
386 and affect community assembly in natural systems (e.g. Aarssen 1997; Lepš 2004). In this
387 study we took advantage of the long-term "Jena Experiment", which is based on a pool of 60
388 grassland species (Roscher et al. 2004). We chose four tall-statured species that had highly
389 productive monocultures and were dominant in mixtures of the Jena Experiment. Thus, our
390 additional experiment complements the so-called "dominance experiment" in the framework
391 of the Jena Experiment. In contrast to our additional experiment, the species pool for the
392 "dominance experiment" was selected on the criterion to consist of potentially dominant
393 species in semi-natural grasslands of the study region (Roscher et al. 2004), but these species
394 showed different levels of monoculture productivity (Roscher et al. 2005, 2007).
395 The main results of our additional experiment on the site of the Jena Experiment were that
396 even tall and dominant species reach higher productivity in mixtures than expected from their
397 monocultures. However, our experimental results clearly indicated that resource availability
398 had strong impacts on diversity effects among these species.

399

400 **Selection effects among tall and dominant grassland species**

401 While complementary resource use and facilitation (quantified as positive "complementarity
402 effects" in the additive partitioning method of Loreau and Hector (2001)) have been identified
403 as the mechanism behind overyielding in numerous biodiversity experiments, the contribution
404 of selection effects ranges from negative (van Ruijven and Berendse 2003) to positive effects
405 (e.g. Dimitrakopoulos and Schmid 2004; Roscher et al. 2005). In line with our hypothesis 1,
406 selection effects were small compared to complementarity effects, and they were negative in
407 the control and positive under shading and fertilization. The small magnitude of selection

effects (Fig. 1f) indicated that no single species reached higher biomass production in the mixtures at the expense of others. Together with the lack of positive transgressive overyielding (Fig. 1c), these results suggest that our experimental species were comparable in their competitive strength as mixture species. Nevertheless, we also found evidence for the influence of a single species, namely *D. glomerata*, on selection effects. *Dactylis glomerata* appeared to benefit most by the supply of additional nutrients; the species had the most productive monoculture and accelerated positive selection effects under fertilization. Interestingly, *D. glomerata*, which also had the most productive monoculture under shading, decreased the generally positive selection effects in this treatment. Thus, it is likely that growth was most controlled by the reduced light supply through shading and not by species interactions. In the control, *D. glomerata* had the least productive monoculture. Selection effects became less negative in mixtures with this species, probably through the use of additional nitrogen provided by N_2 fixing legumes.

Complementarity effects among tall and dominant grassland species

In line with our expectations (hypotheses 2 to 4) we found that complementarity effects varied greatly dependent on resource availability (Fig. 1e), which was consistent with results of RYT analyses (Fig. 1b). In the control without resource manipulation complementarity effects were positive confirming hypothesis 2. In many biodiversity experiments, the positive interactions between N_2-fixing legumes and non-N_2-fixing species contributed to a large extent to positive complementarity effects (e.g. Spehn et al. 2005; Marquard et al. 2009). In the design of our experiment it was unavoidable that all mixtures with exception of the two-species mixture of *A. elatius* and *D. glomerata* contained legumes. Although leaf $\delta^{15}N$ values were above zero in both legumes and all resource treatments, legumes mostly had more depleted $\delta^{15}N$ and higher shoot N concentrations than grasses suggesting that legumes at least partly relied on symbiotic N_2 fixation (Fig. 3b).

Confirming our hypothesis 3, complementarity effects became negative under fertilization. Nyfeler et al. (2009) also reported that positive complementarity effects and transgressive overyielding in four-species grass–legume mixtures disappeared at very high levels of N fertilization (450 kg N ha^{-1} $year^{-1}$). Negative complementarity effects under fertilization were greatest in the mixture of the two grass species (–685 g m^{-2}); average complementarity effects across the mixtures with legumes were –144 (±57) g m^{-2}. Differences in leaf $\delta^{15}N$ between grasses and legumes were smaller under fertilization than in the control (Fig. 3b). It is well known that the physiologically costly symbiotic N_2 fixation is suppressed when mineral soil N availability is increased through fertilization (Hartwig 1998). Analyses of species-level relative yields showed that under fertilization no single species reached higher RYs in biomass production than expected from monocultures, and the legume *O. viciifolia* even underyielded (Fig. 2b).

In contrast to our expectations (hypothesis 4), complementarity effects were positive under shading (Fig. 1f) and none of the species underyielded in this treatment (Fig. 2b). It is likely that nutrients were taken up and used for growth less completely under shading than in the other treatments. In contrast to the control and under fertilization, mineral soil nitrogen concentrations were higher and not depleted to lower levels in the mixtures than expected from the monocultures (Fig. 4). Leaf $\delta^{15}N$ of legume species under shading were higher than in the control and differences to the leaf $\delta^{15}N$ of grass species were smaller (Fig. 3b) suggesting a lower reliance of legumes on symbiotic N_2 fixation. A greater availability of mineral soil N in this treatment due to limited plant growth and therefore lower consumption of soil N or increased soil N mineralization rates at higher soil moisture (Wang et al. 2006) is a possible explanation that legumes were less dependent on symbiotic N_2 fixation in this treatment. Furthermore, higher species-level shoot N concentrations and lower biomass production under shading indicated that plant growth of all species was carbon limited. Plotting shoot N vs. shoot C, which showed that high shoot N concentration occurred at lower

shoot C concentrations in the shading treatment compared to the other treatments (Fig. 5), also supported the view that growth was carbon limited in this treatment (Stitt and Schulze 1994). Undoubtedly the severe shading is not a realistic scenario for tall-growing grassland species under natural conditions, so limited ability to tolerate such shade may be expected in the chosen species. The unexpected positive complementarity effects under shading, however, imply that plant growth was not completely controlled by external resource supply. Possibly, a more diverse canopy structure of plant mixtures compared to the monocultures allowed for a complementary and more complete use of the available light in the mixtures.

Our experiment only addressed short-term changes in community responses to manipulation of resource availability. Several long-term grassland biodiversity experiments have shown that the strength of diversity–productivity relationships and positive complementarity effects increase through time, while selection effects are decreasing (Fargione et al. 2007, Marquard et al. 2009). It also has been observed that diversity effects among the sown species in fertilized and unfertilized experimental grasslands showed great variation during a three-year study period (Lanta and Lepš 2007). Transient dynamics following a perturbation such as environmental changes may differ from conditions when communities reach equilibrium (Tilman 1988). Thus, we cannot exclude that the observed effects of fertilization or shading in a single year of treatment application would change over a longer time span. Nevertheless, our short-term experiment clearly demonstrated that selection effects are small among highly productive and dominant grass and legume species, while the strength and direction of complementarity effects varies greatly dependent on resource availability.

Acknowledgements

This study was part of the Jena Experiment and supported by the German Science Foundation (RO2397/1 within FOR456). We acknowledge A. Weigelt for field coordination. We thank U.

Gerighausen, S. Ferber, C. Kups, the gardeners and several student helpers for their support with the establishment and management of the 4-species-experiment and during sampling. We acknowledge I. Hilke, S. Matthei and H. Geilmann for conducting chemical and stable isotope analyses. We thank F. Voigt and B. Schlöffel for the construction of the shade roof as well as K. Hippler, M. Hertel and A. Fastnacht for the set-up and maintenance of the weather station.

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Table 1 Summary of mixed-effects model analyses of community biomass production, Relative Yield Totals (RYT) or non-transgressive overyielding (D_{mean} = RYT-1), transgressive overyielding (D_{max}), net diversity effects, complementarity effects and selection effects calculated for biomass production in mixtures

Source of variation	Community biomass		Relative Yield Totals		D_{max}	
	Chi^2	p	Chi^2	p	Chi^2	p
Resource	**81.20**	**<0.001**	**36.72**	**<0.001**	**18.64**	**<0.001**
Species richness (SR)	**4.91**	**0.027** ↑	3.25	0.071	0.13	0.714
Resource x SR	**6.11**	**0.047**	0.10	0.950	0.10	0.950
Arrhenatherum elatius (Ae)	0.18	0.672	0.04	0.833	0.12	0.733
Resource x Ae	3.69	0.158	0.64	0.725	0.78	0.676
Dactylis glomerata (Dg)	0.01	0.929	**10.10**	**0.001** ↓	**11.93**	**0.001** ↓
Resource x Dg	0.18	0.913	**9.62**	**0.008**	**15.23**	**<0.001**
Medicago x *varia* (Mv)	0.02	0.875	2.66	0.103	3.31	0.069
Resource x Mv	0.69	0.710	3.87	0.144	5.67	0.059
Onobrychis viciifolia (Ov)	1.41	0.234	2.26	0.133	1.04	0.309
Resource x Ov	**7.83**	**0.020**	0.15	0.926	1.07	0.586

Source of variation	Net diversity effects		Complementarity effects		Selection effects	
	Chi^2	p	Chi^2	p	Chi^2	p
Resource	**25.36**	**<0.001**	**26.92**	**<0.001**	**16.65**	**<0.001**
Species richness (SR)	3.13	0.077	3.32	0.068	0.32	0.572
Resource x SR	0.35	0.839	0.13	0.937	4.64	0.098
Arrhenatherum elatius (Ae)	0.12	0.727	0.02	0.892	0.54	0.461
Resource x Ae	0.96	0.619	1.30	0.523	0.65	0.722
Dactylis glomerata (Dg)	**5.78**	**0.016** ↓	**5.89**	**0.015** ↓	0.03	0.862
Resource x Dg	**11.99**	**0.002**	**12.20**	**0.002**	**11.24**	**0.004**
Medicago x *varia* (Mv)	1.14	0.286	1.03	0.309	0.02	0.898
Resource x Mv	3.04	0.219	2.89	0.236	2.26	0.324
Onobrychis viciifolia (Ov)	2.73	0.099	2.33	0.127	0.12	0.733
Resource x Ov	0.91	0.636	1.54	0.463	3.19	0.203

Models were fitted by stepwise inclusion of fixed effects. Shown are the results of likelihood ratio tests (Chi^2) that were applied to assess model improvement and the statistical significance of the fixed effects (p values). Note that the presence-absence of single species and their interactions with resource availability were fitted in separate models. Significant effects are marked in bold. Arrows indicate increase (↑) or decrease (↓) of the variables in response to increased species richness or the presence of particular species.

Figure captions

Fig. 1 (a) Community biomass production, (b) RYT (or non-transgressive overyielding, D_{mean} = RYT-1), (c) D_{max} (transgressive overyielding), (d) net diversity effects (NE), (f) complementarity effects (CE), and (g) selection effects (SE) as a function of sown species richness. Shown are means (± 1SE) per species-richness level and treatment (control, fertilization, shading). Results of tests for overall means of RYT ≠ 1 and D_{max}, NE, CE and SE ≠ 0, respectively, for each resource treatment are indicated with ns = non-significant, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

Fig. 2 Species-level (a) biomass production, and (b) relative yields (RYs) as means (± 1SE) per treatment (control, fertilization, shading). Species biomass in mixtures was corrected for sowing proportions (i.e. multiplied by species number) before averaging. Significant deviation of the observed from the expected values for species-level relative yields (i.e. RY ≠ 1) across all species-richness levels are indicated with * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$, where RY > 1 indicates overyielding. Results of Tukey`s test applied to test for significant differences in biomass production and relative yields among species for each resource treatment are indicated with letters.

Fig. 3 Species-level (a) shoot nitrogen concentrations, and (b) leaf $\delta^{15}N$ as means (± 1SE) per resource treatment (control, fertilization, shading). Results of Tukey`s test applied to test for significant differences in biomass production, N pools and N concentrations among species for each resource treatment are indicated with letters.

Fig. 4 (a) Soil mineral nitrogen concentrations, and (b) D_{mean} of soil mineral nitrogen concentrations as a function of sown species richness. Shown are means (± 1SE) per species-richness level and treatment (control, fertilization, shading). $D_{mean} < 0$ indicates lower mineral soil nitrogen concentrations in mixture compared to the biomass-weighted mean of the component monocultures. The significance of $D_{mean} \neq 0$ across all species-richness levels was tested separately for each resource treatment, where ns = non-significant, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

Fig. 5 Shoot nitrogen concentrations plotted against shoot carbon concentrations of the four experimental species in each resource treatment (control (C), fertilization (F), shading (S)).

Figure 1

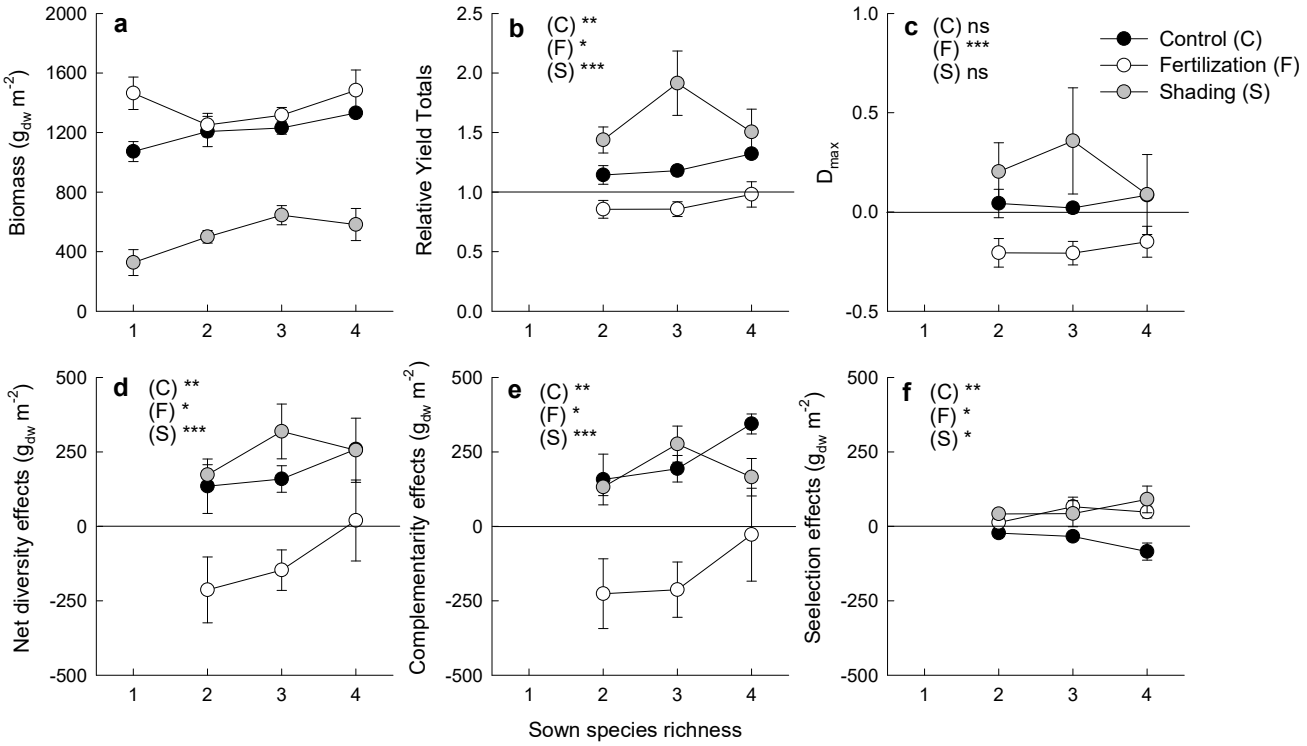

a
b
c
d
e
f
(C) **
(F) *
(S) ***
(C) ns
(F) ***
(S) ns
(C) **
(F) *
(S) *
Control (C)
Fertilization (F)
Shading (S)
Biomass (g_{dw} m^{-2})
Relative Yield Totals
D_{max}
Net diversity effects (g_{dw} m^{-2})
Complementarity effects (g_{dw} m^{-2})
Seelection effects (g_{dw} m^{-2})
Sown species richness

Figure 2

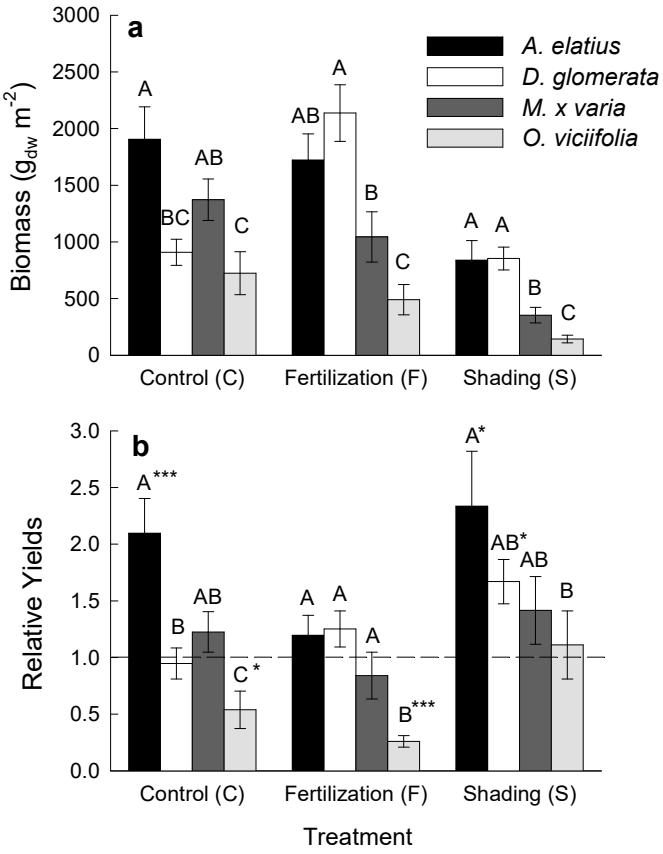

a
A. elatius
D. glomerata
M. x varia
O. viciifolia
Biomass (g$_{dw}$ m^{-2})
3000
2500
2000
1500
1000
500
0
A
BC
AB
C
AB
A
B
C
A
A
B
C
Control (C)
Fertilization (F)
Shading (S)
b
Relative Yields
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A***
B
AB
C*
A
A
A
B***
A*
AB*
AB
B
Control (C)
Fertilization (F)
Shading (S)
Treatment

Figure 3

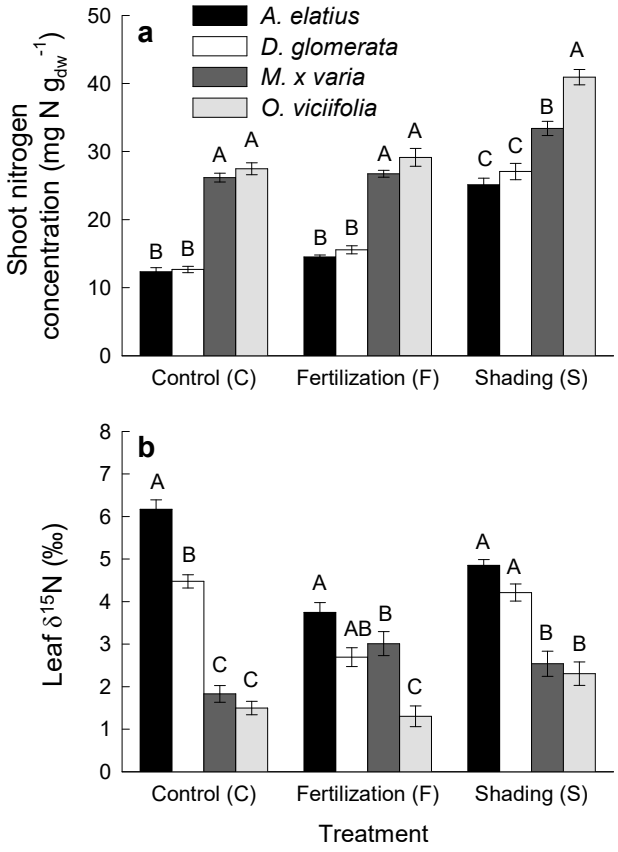

a
A. elatius
D. glomerata
M. x varia
O. viciifolia
Shoot nitrogen concentration (mg N g$_{dw}^{-1}$)
50
40
30
20
10
0
B B A A
B B A A
C C B A
Control (C)
Fertilization (F)
Shading (S)
b
Leaf δ^{15}N (‰)
8
7
6
5
4
3
2
1
0
A B C C
A AB B C
A A B B
Control (C)
Fertilization (F)
Shading (S)
Treatment

Figure 4

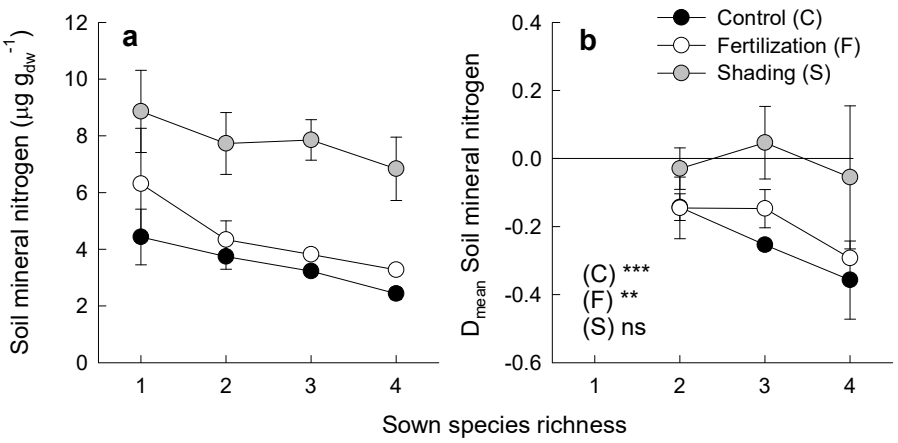
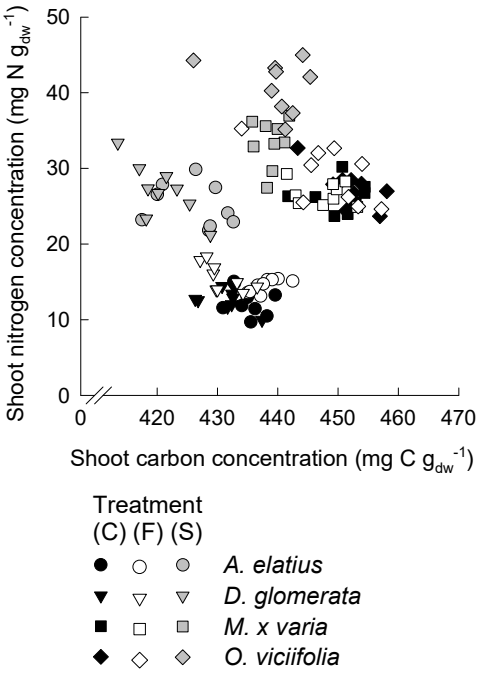

a
b
Soil mineral nitrogen (µg g$_{dw}^{-1}$)
D_{mean} Soil mineral nitrogen
Sown species richness
Control (C)
Fertilization (F)
Shading (S)
(C) ***
(F) **
(S) ns
0
2
4
6
8
10
12
-0.6
-0.4
-0.2
0.0
0.2
0.4
1
2
3
4

Figure 5


Shoot nitrogen concentration (mg N g_{dw}^{-1})
50
40
30
20
10
0
0
420
430
440
450
460
470
Shoot carbon concentration (mg C g_{dw}^{-1})
Treatment
(C) (F) (S)
A. elatius
D. glomerata
M. x varia
O. viciifolia